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Multicentre in-vitro evaluation of the susceptibility of *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* to ciprofloxacin, clarithromycin, co-amoxiclav and sparfloxacin

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Seven laboratories, including a reference laboratory, tested the susceptibility of *Moraxella catarrhalis, Streptococcus pneumoniae* and *Haemophilus influenzae* strains to ciprofloxacin, clarithromycin, co-amoxiclav and sparfloxacin with the Etest. A total of 976 strains were collected. The results with ciprofloxacin and sparfloxacin were consistent for all laboratories, while those with clarithromycin and co-amoxiclav were not. The agreement between Etest MICs and broth microdilution was: ciprofloxacin and sparfloxacin, >95%; clarithromycin for all species, 71–85%; co-amoxiclav for *H. influenzae*, 31%. MIC_{90} values (broth dilution, mg/L) for *M. catarrhalis, S. pneumoniae* and *H. influenzae* were: sparfloxacin, 0.06, 0.5, 0.03; ciprofloxacin, 0.12, 2.0, 0.03; co-amoxiclav, 0.25, 0.25, 0.25; clarithromycin 0.25, 0.25 and 16.

Introduction

Traditionally, ampicillin and amoxycillin have been used for the treatment of respiratory tract infections by *Haemophilus influenzae, Streptococcus pneumoniae* and *Moraxella catarrhalis*. Decreasing susceptibility and emergence of β-lactamase-producing strains have led to the use of alternatives like erythromycin or clarithromycin[1] and fluoroquinolones. We compared the in-vitro activity of sparfloxacin, which shows sufficient activity against pneumococci,[2] with that of ciprofloxacin, co-amoxiclav and clarithromycin against the three common respiratory pathogens. We also wanted to know if the Etest would be suitable for testing *H. influenzae* and *S. pneumoniae* in routine laboratories. Therefore six routine laboratories and a reference one applied the Etest and these results were compared with those obtained by the broth microdilution method, performed by the reference laboratory.

Materials and methods

Bacterial strains

Each of seven laboratories situated in different parts of the Netherlands isolated 45–50 strains each of *H. influenzae, M. catarrhalis* and *S. pneumoniae* from sputum of patients with respiratory tract infections.

Susceptibility testing

Antibiotics used were co-amoxiclav (SmithKline Beecham BV, Rijswijk, The Netherlands), clarithromycin (Abbott BV, Amstelveen, The Netherlands), ciprofloxacin (Bayer BV, Mijdrecht, The Netherlands) and sparfloxacin (Rhone–Poulenc Rorer BV, Amstelveen, The Netherlands).

Strains were grown overnight on appropriate media, suspended in test broth to 0.5 McFarland density and inoculated for confluent growth on to agar media (PDM

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agar with 5% horse blood for *S. pneumoniae* and with 1% haemoglobulin and 1% Isovitalex for *H. influenzae* and *M. catarrhalis*. Etest strips were applied to the plates after the inoculum had dried for 20 min. The concentration ranges of the antibiotics on the strips were co-amoxiclav (amoxycillin/clavulanate 2:1) and clarithromycin, 0.016–256 mg/L; ciprofloxacin and sparfloxacin, 0.002–32 mg/L. Incubation was at 37°C for 24 h. The MICs were read according to the manufacturer's recommendation. Control strains used by all laboratories were *H. influenzae* ATCC 49247 and *S. pneumoniae* ATCC 49619.

Both Etest and broth microdilution MICs[3] were determined for the strains collected by the reference laboratory and the reference strains. The medium used was Isosensitest Broth (Oxoid CM 473), supplemented with 2% lysed horse blood for *S. pneumoniae* and with 0.5% yeast extract (Difco), 0.0015% haemin and 0.0003% NAD for *H. influenzae*. The final inoculum size per microtitre well was 1.5–2.5 $\times$ 10^5 cfu/mL. The plates were incubated at 37°C and growth was assessed after 24 h. The MIC was defined as the lowest concentration preventing visible growth in the test medium.

Results

A total of 976 strains, including *M. catarrhalis* (305), *S. pneumoniae* (320) and *H. influenzae* (351) were tested by Etest; 141 of these strains were tested by both methods. Four per cent of *H. influenzae* and 22% of *M. catarrhalis* strains produced β-lactamase as determined by the nitrocefin method.

For comparison purposes, Etest MICs were raised to the next highest doubling dilution value used in the broth dilution MIC determination. MICs recorded by both methods are given in Table I. The results found with ciprofloxacin and sparfloxacin by the Etest were in the same range for the seven laboratories, but there was a considerable variation with clarithromycin for all strains including the reference strains, and with co-amoxiclav for *H. influenzae*. Six laboratories reported problems with reading of clarithromycin zones (unclear) and two with reading the co-amoxiclav results for *H. influenzae* (double zones).

Comparing the Etest results with those of the broth dilution method for 141 strains (Table II), we found excellent correlation (>95% agreement to within one doubling dilution) with co-amoxiclav for *M. catarrhalis* and *S. pneumoniae* and with ciprofloxacin and sparfloxacin for all species. Significant disagreements (fourfold or greater MIC differences) were found with clarithromycin for all species and with co-amoxiclav for *H. influenzae*.

MICs obtained by the broth dilution method for the reference strains were well within the ranges given by the NCCLS. Assuming the data obtained by the broth microdilution method to be correct, MIC_{90} values and

Table I. MICs and percentage of susceptibility of co-amoxiclav, clarithromycin, ciprofloxacin and sparfloxacin against common respiratory pathogens, determined by Etest by all laboratories (all) and the reference laboratory (ref.) and by the broth microdilution test (broth ref.). Breakpoints of susceptibility used: co-amoxiclav, 0.5 mg/L; clarithromycin, 4 mg/L; ciprofloxacin and sparfloxacin, 1.0 mg/L

	Range (mg/L)			MIC_{90}			% Susceptible		
	Etest		broth	Etest		broth	Etest		broth
	all	ref.	ref.	all	ref.	ref.	all	ref.	ref.
M. catarrhalis									
co-amoxiclav	0.016–2	0.016–1	0.016–0.5	0.25	0.25	0.25	97	98	100
clarithromycin	0.017–>64	0.016–>64	0.064–16	8	8	0.25	89	78	95
ciprofloxacin	0.002–2	0.008–2	0.004–4	0.125	0.125	0.125	99	98	95
sparfloxacin	0.002–0.5	0.008–0.5	0.004–0.5	0.064	0.064	0.064	100	100	100
S. pneumoniae									
co-amoxiclav	0.016–4	0.016–4	0.016–4	0.032	0.032	0.25	99	98	98
clarithromycin	0.016–>64	0.016–>64	0.016–>64	1	2	0.5	93	90	90
ciprofloxacin	0.125–32	0.5–32	0.25–4	2	2	2	74	66	69
sparfloxacin	0.016–1	0.125–1	0.125–0.5	0.5	0.5	0.5	100	100	100
H. influenzae									
co-amoxiclav	0.032–>64	0.064–>64	0.032–4	4	4	0.25	67	75	98
clarithromycin	0.125–>64	1–>64	0.125–32	16	32	16	31	17	46
ciprofloxacin	0.002–8	0.004–0.064	0.004–0.064	0.032	0.032	0.032	99	98	100
sparfloxacin	0.002–2	0.004–0.064	0.004–0.064	0.016	0.032	0.032	99	98	100

Table II. Etest compared with broth microdilution: number of strains with differing MIC values

Antibiotic	Dilution factor ⩾−3	−2	−1	0	+1	+2	⩾+3	Discrepancy ⩾2 dilutions
M. catarrhalis (45)								%
co-amoxiclav			3	17	23	2		5
clarithromycin			10	25	2	1	6	16
ciprofloxacin			5	38	2			0
sparfloxacin			1	40	4			0
S. pneumoniae (49)								
co-amoxiclav		2		47				4
clarithromycin	1	7	22	5	3	1	5	29
ciprofloxacin		1	6	35	6	1		4
sparfloxacin			6	38	5			0
H. influenzae (48)								
co-amoxiclav			11	3	11	17	17	69
clarithromycin			7	21	13	1	6	15
ciprofloxacin				46	2			0
sparfloxacin			3	45				0

percentages of susceptibility were calculated according to the breakpoints used for The Netherlands (Table I).

Discussion

Most experience with Etest has been obtained with susceptibility testing of Gram-negative rods[4] and routine antibiotics. Experience with other organisms and newer drugs is limited.[5]

Like Pankuch *et al.*[6] we found the Etest to be accurate in testing co-amoxiclav susceptibility of *S. pneumoniae* and in testing fluoroquinolones. The variation in results with ciprofloxacin and sparfloxacin between the seven laboratories was minimal. We found that sparfloxacin was four times more active *in vitro* than ciprofloxacin, which confirmed the data of Piddock[7].

The variations in results with clarithromycin and co-amoxiclav were considerable: 5–20% of *M. catarrhalis*, 0–16% of *S. pneumoniae* and 39–82% of *H. influenzae* were recorded as resistant to clarithromycin; some laboratories found all strains of *H. influenzae* susceptible, others >90% resistant to co-amoxiclav. The discrepancies may be caused by variation in susceptibility of isolates in some centres or by methodological problems. The latter is most likely. Interpreting indistinct endpoints by double zones with co-amoxiclav may have influenced the Etest results for *H. influenzae*,[8] recording only 67% susceptibility versus 98% with the broth dilution method, the latter fitting well with the β-lactam resistance among *H. influenzae* strains in The Netherlands (<5%). We concluded, therefore, that the Etest results for co-amoxiclav were unreliable.

Unclear zones hampered the correct reading of clarithromycin results. The high inoculum required for Etest, 10^7 cfu compared with 10^5 cfu for broth microdilution, might also have influenced the results[9] as well as the slow growth in the case of *H. influenzae*. MIC_{90} values of clarithromycin for *M. catarrhalis* and pneumococci of >0.25 mg/L[9,10] are exceptional. Unusually high MICs were found by all laboratories, therefore we concluded that routine testing of clarithromycin with the Etest is risky.

The first choice for treatment of acute bacterial exacerbations of chronic bronchitis in The Netherlands is amoxycillin or co-amoxiclav. Since we have found that co-amoxiclav still has a high activity against a high percentage of all pathogens, we think there is no need to change this policy as yet.

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